Consommation et Corporations Canada
Consumer and Corporate Affairs Canada

Bureau des brevets
Patent Office

Ottawa, Canada
K1A 0C9

(11) 2,008,114

(22) 1990/01/19

(43) 1990/07/20

(52) 402-522
C.R. CL. 167-206
99-1
99-83

5,037,7/08

(51) INTL.CL.[5] C08F-8/02; C08F-26/02; A61K-31/74; A23L-2/26

(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Alkylated Polyethylenimine Derivatives, Process for their Preparation, their Use as Pharmaceuticals and Pharmaceutical Preparations

(72) Heitz, Walter - Germany (Federal Republic of) ;
Fischer, Thomas - Germany (Federal Republic of) ;
Kerekjarto, Bela - Germany (Federal Republic of) ;

(73) Hoechst Aktiengesellshaft - Germany (Federal Republic of) ;

(30) (DE) P 39 01 527.0 1989/01/20

(57) 8 Claims

Canada

Abstract of th[redacted]isclosure: HOE [redacted]F 018

Alkylated polyethylenimine derivatives, process for their preparation, their use as pharmaceuticals and pharmaceutical preparations

Novel non-crosslinked and crosslinked alkylated polyethylenimines are described which can be used as hypolipidemic agents in the light of their bile acid-binding activity. A process for the preparation of the said polyethylenimines and pharmaceutical preparations are also described.

Description

Alkylated polyethylenimine derivatives, process for their preparation, their use as pharmaceuticals and pharmaceutical preparations

The invention relates to alkylated polyethylenimine derivatives, to a process for their preparation, to pharmaceutical preparations based on these compounds and to their use as pharmaceuticals, in particular for lowering increased lipid levels.

Insoluble basic, crosslinked polymers have been used for a considerable time for binding bile acid and are used therapeutically in the light of these properties. Chologenic diarrhea (for example after resection of the ileum) and increased cholesterol blood levels are treated causally as the object of therapy. In the latter case, it is a matter of intervention in the enterohepatic circulation, the corresponding resynthesis of cholesterol in the liver being provoked in place of the bile acid component taken out of the circulation. Recourse is made to the circulating LDL (low density lipoprotein) cholesterol to meet the cholesterol need in the liver, the hepatic LDL receptors coming into effect in increased number. The acceleration of LDL catabolism thus caused has an effect owing to the reduction of the atherogenic cholesterol content in the blood.

The ion exchangers used as pharmaceuticals have either quaternary ammonium groups (such as colestyramine) or secondary or tertiary amine groups (such as colestipol) as active groups. The daily dose of colestyramine is expediently 12-24 g, and 32 g are recommended as the highest daily dose. 15-30 g is the recommended daily colestipol dose. Taste, odor and high dosage make patient compliance more difficult. The side effects go back to lack of selectivity (for example avitaminoses), which

even have to be considered in the dosage of medicaments given simultaneously, and also to bile acid depletion, which cause various gastrointestinal disturbances (constipation, steatorrhea) to a different degree. For both preparations, a therapeutic significance by combination with other hypolipidemic pharmaceuticals such as fibrates, HMG-CoA reductase inhibitors, probucol (cf., for example, M.N. CAYEN, Pharmac. Ther. 29, 187 (1985) and 8th International Symposium on Atherosclerosis, Rome, Oct. 9-13, 1988, Abstracts p. 544, 608, 710) has been described, the effects obtained even making the therapy of severe hyperlipidemia possible. It therefore appears significant to find suitable substances with the given principle of action and without the disadvantages of the preparations presently used.

The following features of the preparations mentioned and, in particular, of colestipol are regarded as worthy of improvement:

1. The high daily doses, which are to be put down to a relatively low binding rate at neutral pH in isotonic medium and the release (partial) of the adsorbed bile acid again.

2. The qualitative shift in the bile acid composition of the bile with a decreasing tendency for chenodeoxycholic acid and the increasing risk of cholelithiasis associated with this.

3. The lack of a damping effect on the cholesterol metabolism of the intestinal bacteria.

4. The binding rate of vitamins and pharmaceuticals, which is too high, makes a need for substitution of these substances and for blood level controls necessary in some cases.

5. A further improvement can be obtained in the form

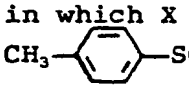
for administration.

The removal of the deficiencies listed is surprisingly achieved by the use of high molecular weight alkylated polyethylenimines. The non-absorbable macromolecules exhibit their action both in soluble form, corresponding to the non-crosslinked structure, and in the insoluble state as the crosslinked polymer.

Crosslinked polyethylenimines are described in US Patent 3,332,841. The crosslinking is carried out, inter alia, by means of alkylene groups having 2 to 8 carbon atoms, the molecular weight of the starting polymers being between 800 and 100,000. For the treatment of temporary hyperacidity of the stomach, 0.25 to 5 g are administered per dosage unit. Neither the binding of bile acid nor a lipid-lowering activity of the crosslinked polyethylenimines associated with this is described, as without alkylation the polyethylenimines have no binding capacity or only an insignificant binding capacity compared to the gallic acids, depending on the type. Owing to the large potential charge density, provision can be made for sufficient binding capacity by means of alkylation and for affinity and binding specificity by means of the choice of substituents of appropriate hydrophilic/hydrophobic character.

The invention therefore relates to non-crosslinked and crosslinked alkylated polyethylenimines, wherein the starting polyethylenimine has a molecular weight of 10,000 to 10,000,000, the alkylating agent has the formula I

$$R\text{-}X \qquad (I)$$

in which X is chlorine, bromine, iodine, $CH_3\text{-}SO_2\text{-}O\text{-}$ or  and

R is a straight-chain or branched $C_1\text{-}C_{30}$-alkyl

radical which is optionally substituted by a mono- or bicyclic saturated hydrocarbon having 5 to 10 ring carbon atoms, or by a phenyl radical

and, in the case of the crosslinked alkylated polyethylenimines, the crosslinking agent is an α,ω-dihaloalkane having 2-10 carbon atoms or a higher functionalized haloalkane having 2-10 carbon atoms.

The process for the preparation of the alkylated polyethylenimine derivatives according to the invention comprises alkylating a polyethylenimine having a molecular weight between 10,000 and 10,000,000 with an alkylating agent of the formula R-X, in which X and R have the meanings indicated, and, if desired, crosslinking with an α,ω-dihaloalkane having 2-10 carbon atoms or a higher functionalized haloalkane having 2-10 carbon atoms by methods customary in polymer chemistry.

The crosslinking can be carried out before or after the alkylation. Carrying out the crosslinking and the alkylation simultaneously is particularly preferred.

Polyethylenimines having a molecular weight above 100,000 are preferably employed.

In the alkylating agents R-X, X is preferably chlorine or bromine.

R is preferably a primary alkyl radical. If the alkyl radicals are substituted by the ring systems mentioned, these are preferably arranged so that they are linked to the polyethylenimine via a spacer having 1 to 4 CH_2 groups. The cyclohexyl radical is particularly suitable as a monocyclic saturated substituent. A suitable bicyclic hydrocarbon radical is, for example, decalin. A particularly suitable alkylating agent, whose alkyl radical is substituted by phenyl, is benzyl bromide. A suitable alkylating agent without substituents in the alkyl radical is preferably butyl chloride.

The alkylation can be carried out in several stages. In this way, the possibility exists of fixing different substituents to the same polymer.

The ratio of the alkylating agent employed to the amino groups of the polyethylenimine is 0.2:1 to 5:1, preferably 0.5:1 to 2:1.

By means of the reaction with alkylating agents, a part of the secondary amino groups in the chain are converted into tertiary and quaternary structures. The formation of tertiary amino groups is preferred.

Suitable crosslinking agents are, for example, di- and trihaloalkanes, preferably α,ω-dihaloalkanes such as, for example, 1,6-dibromohexane and 1,10-dibromodecane. The amount of the crosslinker is preferably 2-25 mol-%, relative to the alkylating agent employed.

The alkylated polyethylenimines according to the invention adsorb acids intrinsic to the body, in particular gallic acid. In the light of these properties, they are in a position to lower elevated cholesterol levels. The alkylated polyethylenimines according to the invention have essentially more favorable bile acid-absorbing properties compared to colestipol, as is discernable from the experiments described below.

1. In vitro experiments

1.1 Adsorption batch containing individual bile acids
Experimental conditions: volume = 10 ml;temperature: 37°, incubation in a shaking water bath, duration: 2 hours; medium: isotonic buffered physiological saline solution, pH 7.0; bile acid: 10-15 µmoles; adsorber (= compound according to the invention or comparison compound): 10-100 mg.

The bile acid in equilibrium with the adsorbate is determined by means of enzymatic analysis. The methods via 3α-hydroxy- or 7α-hydroxysteroid dehydrogenases (EC 1.1.1.50 or EC 1.1.1.159) were carried out according to the description in Bergmeyer (H.U. Bergmeyer, Methoden der enzymatischen Analyse (Methods of Enzymatic Analysis), 2nd edition (1970), p. 1824) or the product information to product No. H-9506 from SIGMA CHEMICAL Co. (St. Louis, USA). The proportion of bile acids bound was calculated from the difference between the control batches without adsorber and the complete batches. The experiments for the characterization of the adsorber were carried out with variation of the prestated bile acid concentration or the adsorber amount, and less frequently the incubation period, pH or ionic strength.

An alkylated crosslinked polyalkylenimine according to Example 2 showed a qualitatively better effect owing to stronger cholate binding than colestipol since, incubated with a 2 mM glycocholate solution, 50 mg of colestipol adsorbed under 6 % of the bile acid, on the other hand polyimine as in Example 2 absorbed 74 % to 76 % of the bile acid.

1.2 Reversibility testing.

The adsorbate removed from the equilibrium in a batch as described under 1.1 was postincubated with fresh bile acid-free medium and the bile acid released was determined as under 1.1.

In this experiment, it was possible to show that less bile acid was released again from adsorbates containing alkylated crosslinked polyethylenimine as in Example 2 since, from the adsorbate of 50 mg of colestipol with 2 mM of glycocholate, 57 % of the gallic acid was released again, on the other hand

only 4 % to 5 % was released from the adsorbate of 50 mg of compound according to Example 2.

1.3 Adsorption batch containing bile acid mixtures.

The conditions indicated under 1.1 were modified with respect to bile acid such that 40 µmoles of the tauroconjugate of cholate, chenodeoxycholate, deoxycholate and lithocholate were brought into the batch simultaneously, 20 - 100 mg of the adsorber being used. The individual adsorption rate of the bile acid was determined by separation and determination by means of high pressure liquid chromatography (N. Parris, Analyt. Biochem. 100 (1979) 260-263).

Under these experimental conditions, an alkylated polyethylenimine according to the invention as in Example 1 showed a larger binding rate, as 20 mg of colestipol bound 54 % of a mixture of 4 tauroconjugated bile acids (each containing 4 mM), on the other hand polyimine as in Example 1 bound 83 %.

In vivo experiments

Young male Wistar rats of a body weight of about 200 g were divided into groups of 6 animals and kept on standard feed. In each case samples of faeces were taken from the animals for analysis before the start of the experiment, and 1 and 2 weeks after the start of the experiment. If water-soluble, the adsorber was administered daily as a weakly acidic buffered solution at 100, 250 or 500 mg/kg of body weight for 14 days using the stomach tube; as the insoluble substance the adsorber, suspended with 1 % of Tylose(R) (water-soluble cellulose ether) as the vehicle, was incorporated at 250 or 500 mg/kg of body weight daily for 14 days using the stomach tube.

In samples of faeces, the neutral steroids were extracted

after homogenizing with chloroform/methanol 2:1 (v/v), the extract was hydrolyzed and the hydrolyzate was extracted with diethyl ether/heptane 2:1 (v/v). After evaporating the solvent, the sample was subjected to gas chromatographic separation and analysis (H-CH. Curtius and W. Bürgi, Z. Klin. Chemie 4 (1966) 38 - 42).
The examinations of the samples of faeces showed the following:

2.1 If the adsorber was a crosslinked alkylated polyethylenimine as in Example 2, a more rapid onset of action was determined since, after feeding 250 mg of adsorber/kg of body weight daily for 7 days to rats, the additional excretion of unconjugated bile acid in the faeces was 38 % with colestipol, on the other hand it was 115 % with the compound as in Example 2. The effect of polyethylenimine is obtained with colestipol only after 14 days.

2.2 The desired inhibition of the bacterial conversion of cholesterol in the intestine, associated with an additional excretion of cholesterol, is caused to a larger degree by alkylated crosslinked polyethylenimine as in Example 2, since under identical experimental conditions, the cholesterol excretion in rat faeces by colestipol is increased by 28 %, and by 89 % by a compound as in Example 2; at the same time, the coprostanol excretion owing to colestipol changes by +4 %, but by -30 % owing to a compound as in Example 2.

From the experimental results it can be clearly discerned that:
both the non-crosslinked and the crosslinked alkylated polyethylenimines show by means of in vitro adsorption experiments that, compared to colestipol

- the quantity of the bile acid bound is increased by 50-60 % (Examples 1 and 2)
- the binding of cholate is increased 10-12-fold

(Example 2)

- the desorption rate is 10-15-fold lower from bile acid polymer adsorbates (Example 2).

In the rat experiments, it was shown that non-crosslinked polyethylenimines, like crosslinked alkylated polyethylenimines, given orally in the test range up to 500 mg/kg of body weight daily are tolerated without symptoms. It was possible to show advantages compared to identical doses of colestipol, in that

- a more rapid onset of action takes place, whereby the additional bile acid excretion was tripled after one week's use
- the cholesterol elimination was increased three-fold
- the bacterial conversion of cholesterol in the intestine was slowed down and in this way the production and excretion of coprostanol was significantly (= 30 %) reduced (Example 2)

The compounds according to the invention are suitable in the light of their properties for use as pharmaceuticals, in particular for lowering increased lipid levels. The invention therefore also relates to the use as hypolipidemic agent and pharmaceutical agent. In the pharmaceutical agents, polyethylenimines according to the invention can also be present in the form of physiologically tolerated salts with acids.

A particular advantage is the use of crosslinked alkylated polyethylenimines. The crosslinked products can give off no substances into their environment. This is of significance for the development of a non-toxic material.

The dose to be administered daily is preferably 1.0 to 10.0 g, in particular 5 g. It can be divided into several individual doses.

The compounds according to the invention can be converted as such, or after addition of customary auxiliaries, into

forms of preparation for oral administration, such as, for example, tablets, capsules, syrups, aqueous solutions, suspensions etc. In this connection, it may be expedient first to bring active compounds obtained in solid form to a desired particle size, for example, by fine grinding. Suitable auxiliaries are, for example, lactose, starch, gelatin, talc etc. The production of tablets is carried out, for example, by means of moist granulation and subsequent compression.

Moreover, the alkylated polyethylenimines can also be incorporated into foodstuffs such as bread, fruit juice etc. or taken together with foodstuffs.

The compounds according to the invention can also be used in combination with other active compounds. Other active compounds which are suitable are, for example, HMG-CoA reductase inhibitors, vitamins, geriatric agents and antidiabetic agents.

The following examples are intended to illustrate the invention:

Example 1

44.3 g of polyethylenimine (mol. wt. about 1,000,000) are dissolved in 1.05 l of H_2O in a 4 l reaction flask; 191.4 g of butyl chloride are added and the mixture is heated to reflux for 24 hours with vigorous stirring (500 rpm). A turbid viscous reaction mixture is formed. 1 l of 2 N NaOH solution is added to this mixture after cooling and the batch is again brought to reflux for 24 hours. After cooling, two phases form. The organic phase is separated off and freed from the solvent in vacuo. The weight of solid is 75.4 g.
The product has a degree of alkylation of about 50 % and, after addition of equivalent amounts of acid, is water-soluble.

Example 2

44.3 g (1 mol) of polyethylenimine is dissolved in 1.05 l of H_2O in a 4 l reaction flask; 186.6 g of butyl chloride (2 mol) and 48.8 g (0.2 mol) of 1,6-dibromohexane are added and the mixture is heated to reflux for 24 hours with vigorous stirring (500 rpm). A turbid viscous reaction mixture is formed. 1 l of 2 N NaOH solution is added to this mixture after cooling and the batch is brought to reflux again for 24 hours. After cooling the reaction mixture, the solid is filtered off with suction, washed until neutral and dried. The weight is 87.27 g. The product is insoluble in water and can be swollen in methanol.

The ratio of starting compound to alkylating agent and crosslinking agent can be varied within certain limits. Under the reaction conditions indicated, products are then obtained having another degree of alkylation and crosslinking.

Example 3

The reaction mixture from 4.3 g of polyethylenimine, 100 ml of water and 29.3 g of 1-chloro-2-cyclohexylethane is heated to reflux for 24 hours, a yellowish turbid mixture being formed. After adding 100 ml of 2 N NaOH solution, the mixture is heated to reflux again for 24 hours. The reaction mixture forms a two-phase system. The organic phase is separated off and freed from the solvent on a rotary evaporator. 15.34 g of a highly viscous material are obtained.

Example 4

A mixture of 88.6 g of polyethylenimine (50 percent in water; $\hat{=}$ 1 mol), 186.6 g of n-butyl chloride and 48.8 g of 1,6-dibromohexane in 2 l of water is heated to 80°C

for 24 hours under 10 bar of nitrogen in a stirring or shaking autoclave. The protective gas is replaced by 7 bar of ammonia and the mixture is again heated to 80°C for 24 hours.

After cooling the reaction mixture, the precipitate formed is filtered off with suction and washed with water until neutral. The product is washed with methanol and eluted in a column using about 2.5 l of methanol, about 1.5 l of 2 N acetic acid, about 2 l of -2 N ammonia water and finally about 2 l of methanol. After filtering off with suction, the product is dried in vacuo at a maximum of 50°C.

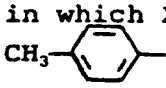
Yield 78 g.

The product can be swollen in various solvents, but is insoluble.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A non-crosslinked or crosslinked alkylated polyethylenimine, wherein the starting polyethylenimine has a molecular weight of 10,000 to 10,000,000, the alkylating agent has the formula I

$$R\text{-}X \qquad (I)$$

in which X is chlorine, bromine, iodine, $CH_3\text{-}SO_2\text{-}O$- or  and

R is a straight-chain or branched C_1-C_{30}-alkyl radical which is optionally substituted by a mono- or bicyclic saturated hydrocarbon having 5 to 10 ring carbon atoms, or by a phenyl radical

and, in the case of the crosslinked alkylated polyethylenimines, the crosslinking agent is an α,ω-dihaloalkane having 2-10 carbon atoms or a higher functionalized haloalkane having 2-10 carbon atoms.

2. A process for the preparation of non-crosslinked or crosslinked alkylated polyethylenimine derivatives, which comprises alkylating a polyethylenimine having a molecular weight between 10,000 and 10,000,000 with an alkylating agent of the formula R-X, in which X and R have the meanings indicated and, if desired, crosslinking with an α,ω-dihaloalkane having 2-10 carbon atoms or higher functionalized haloalkane having 2-10 carbon atoms by methods customary in polymer chemistry.

3. The process as claimed in claim 2, wherein one or more of the following measures are observed:

a) polyethylenimines having a molecular weight above 100,000 are employed,

b) in the alkylating agents R-X, X is chlorine or bromine,

c) in the alkylating agents, R is a primary alkyl radical which is optionally substituted by a cyclo-

hexyl, decalin or phenyl radical, where these substituents are arranged such that they are linked to the polyethylenimine via a spacer having 1 to 4 CH_2 groups,

d) the ratio of the alkylating agent employed to the amino groups of the polyethylenimine is 0.5:1 to 2:1,

e) the crosslinking agent is 1,6-dibromohexane or 1,10-dibromodecane,

f) alkylation and crosslinking are carried out simultaneously.

4. A pharmaceutical preparation which contains a compound as claimed in claim 1 or its physiologically tolerated salt with an acid.

5. A method for the production of a pharmaceutical preparation, which comprises converting a compound as claimed in claim 1 into a suitable form for administration.

6. The use of a compound as claimed in claim 1 as a hypolipidemic agent.

7. The use of compounds as claimed in claim 1 as an additive in foodstuffs and fruit juices.

8. The non-crosslinked or crosslinked alkylated polyethylenimine as claimed in claim 1, and substantially as described herein.